

The University of Chicago

THE REPRODUCTIVE SYSTEM
OF THE MALE OPOSSUM, DIDELPHIS
VIRGINIANA KERR, AND ITS EX-
PERIMENTAL MODIFICATION

A DISSERTATION SUBMITTED TO THE FACULTY
OF THE DIVISION OF THE BIOLOGICAL
SCIENCES IN CANDIDACY FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

DEPARTMENT OF ZOOLOGY

1937

By
ELIZABETH BROWN CHASE

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THE REPRODUCTIVE SYSTEM OF THE MALE OPOSSUM, DIDELPHIS VIRGINIANA KERR AND ITS EXPERIMENTAL MODIFICATION ¹

ELIZABETH BROWN CHASE

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ONE TEXT FIGURE AND ONE PLATE (SEVEN FIGURES)

INTRODUCTION

The opossum, *Didelphis virginiana*, was chosen for the present study because it is an animal which reproduces throughout most of the year and because, as a marsupial, it might show conditions and reactions differing from those of placental mammals. The general morphology and physiology of the male reproductive system of the opossum have been little studied, although numerous accounts of the female anatomy are present in the literature, and the physiology of the female reproductive system has been studied extensively by Hartman. The male accessory reproductive glands have been of especial interest in this study from several standpoints: 1) what structures are actually present, 2) their histological and cytological characteristics, and 3) any particular advantage they may offer in the study of the internal secretion of the testis. The normal morphology and histology of the male system were studied with these points in mind. As an aid in the interpretation of the adult conditions, the development of the male reproductive system was investigated by a study of pouch young of different ages.

Following detailed study of the normal male conditions, an introductory investigation of the physiology of the re-

¹ This investigation has been aided by a grant from the Rockefeller Foundation to The University of Chicago.

productive system was conducted. Castrations were performed to see if detectable changes occur in the accessory glands. The effect of addition of hormone was studied by administration of gonadotropic agents, prospermin and Antuitrin-S into semi-castrate animals.²

MATERIALS

The material used in this study consisted of forty-three adult male opossums of the species *Didelphis virginiana* Kerr. Three of these had been preserved in formalin for several years and the date of collection and of killing were unknown. Five were captured in North Carolina during the winter months and kept in outdoor cages there. The remainder were received from Kentucky, Oklahoma and Missouri during the fall and winter months. They were kept in the laboratory at The University of Chicago for varying lengths of time, from a few days to 6 months.

In the study of the development of the parts of the male urogenital system, thirty-eight pouch young opossums collected in Texas and Oklahoma were studied in serial section. The ages ranged from 13 days to 89 days, calculated from lengths according to the growth chart by Hartman ('28).

NORMAL CONDITION

The gross morphology of the male reproductive system of the opossum was first described by Cowper (1704). Since that time a few additional studies have been made, notably those of Leydig (1850), Oudemans (1892), Disselhorst ('04), and v. d. Broek ('10 a, '33). Oudemans and Disselhorst in-

² This problem was suggested by Dr. Carl R. Moore, whose advice and encouragement during the course of the work have been invaluable. The author is greatly indebted to Dr. Eleanor Conway and Dr. David Bodian for assistance with some of the problems encountered. A large number of the animals used in this study were secured through the courtesy of the anatomy department of The University of Chicago, and many of the pouch young examined have been kindly provided by Dr. Carl G. Hartman. I wish to thank Dr. J. A. Morrell of E. R. Squibb and Sons and Dr. Oliver Kamm of Parke, Davis and Company, for their co-operation in furnishing prospermin and Antuitrin-S for experimental treatment.

clude brief histological descriptions of some parts, but complete histological study is lacking.

Gross anatomy

The reproductive system of the opossum differs in several respects from that of placental mammals. The scrotum is prepenial and is attached to the body by a narrow stalk. The scrotum is definitely bilobed, with a mass of connective tissue dividing the two sides internally.

The testes and epididymides are similar in gross anatomy to those of other mammals. The vas deferens leads from the tail of the epididymis, along the body of the epididymis and through the scrotal stalk. In the body wall, it then proceeds laterad, around the epipubic bones and into the body cavity. It extends then medially to enter the dorsal wall of the urethra a short distance posterior to the neck of the bladder.

In the animals examined, the urethra varied widely in length and diameter. In some, the urethra was a narrow organ, of a similar color and diameter throughout, and running straight from the bladder to the cavernous urethra. In other animals, the urethra was thickened except at the most caudal end where an abrupt change in diameter was seen. In these specimens, the urethra was curved instead of straight as described for the first type. The curvature may be slight, as shown in figure 1, moderate as in the figures by Cowper (1704) and Oudemans (1892), or may be extreme. In one specimen examined, the urethra made a complete loop in the course from the bladder to the cavernous portion of the urethra. That these differences in size of the urethra are associated with age was suggested by Oudemans (1892), who pointed out that the narrow straight type was found in pouch young, while the enlarged urethra was found in adult animals.

In a large urethra, striking differences in diameter of the cranial and caudal parts and the structure of these regions leads to a division of this portion of the urethra into two general parts, which have been called glandular and mem-

branous (fig. 1). The thick glandular portion shows three distinct regions, differing in color and diameter. The first part of the urethra, designated prostate I in figure 1, is orange or brownish gray in color in fresh specimens. The second portion of the urethra, prostate II in figure 1, is a light pink and is more solid than the parts anterior and posterior to it. The diameter of this region is the greatest

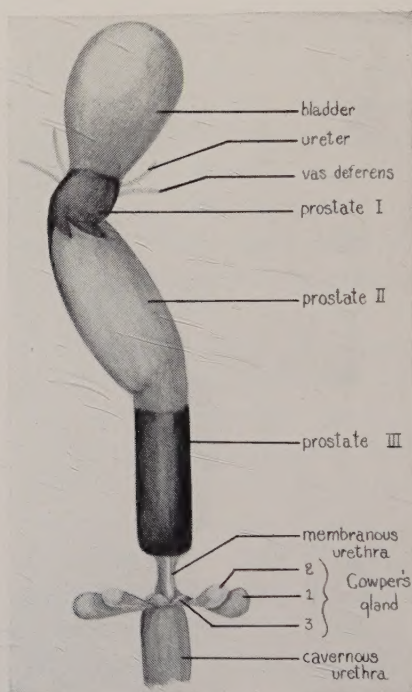


Fig. 1 Urethra and associated structures of male opossum. $\times \frac{3}{2}$.

found in the entire urethra. A comparatively straight line marks the separation of this region from the one following. The third part, prostate III, figure 1, is a dark gray green color and somewhat translucent. It is longer than either of the other two regions and less in diameter. Cross sections of fresh material show that the colors of all three parts extend half way to the lumen. Although these differences have

not been reported for *Didelphis virginiana*, Martin and Jones (1834) noted them in *Didelphis azarae* Temm. Numerous small pits which mark the openings of the collecting ducts of the glands can be found in the folds of the urethral lumen. The radial arrangement of the gland tubules can be seen with the naked eye in cut edges of the urethral wall.

The narrow membranous portion of the urethra extends from the abrupt ending of the glandular region to the point where Cowper's glands come in contact with it. From that point on, the cavernous portion of the urethra is found. A detailed description of this region of the urethra is unnecessary in the present study.

Cowper's glands are present in the opossum as three paired lobes. The largest of these (Cowper's 1, fig. 1) is hidden beneath a large amount of fatty tissue lateral and dorsal to the urethra. When well developed, it is a large, rather clear lobular structure with a definite duct leading to the wall of the urethra. The other two lobes of Cowper's glands are smaller than Cowper's 1 and are enclosed in a common muscular capsule. When large, the two can be distinguished easily by means of a constriction between them, but when small, a separation can be seen only by microscopical examination of serial sections. Cowper's 2 (fig. 1), the larger and more lateral of the two lobes, is a flattened, spade-shaped structure closely applied to the anterior face of the bulbus cavernosus urethralis. The duct of this lobe cannot be found without histological examination as it extends beside the third lobe and is enclosed by the thick sheath of muscle which surrounds the smaller lobe. The third lobe (Cowper's 3, fig. 1) is a flattened structure, rather leaf-shaped, with the wide portion at the median line of the urethra. The ducts of all three parts join and run parallel to the lumen of the urethra as a series of sinuses before opening into it.

Histology

Testis. The histology of the testis is essentially the same as in other mammals. All the animals examined showed

spermatozoa in the testis tubules, except number 39, an animal killed the early part of December, in which no fully formed spermatozoa were found.

In studies of pouch young, the sexes can be distinguished externally at about $1\frac{1}{2}$ weeks after birth (McCrady, '38). In the 13-day-old specimens examined, the male and female gonads were readily distinguishable histologically. At 25 days, the testes have begun to descend and are found in the connective tissue of the ventral body wall. The descent continues until they are entirely within the scrotum at 89 days.

Vas efferens. The marsupials differ from other mammals in having only a single efferent duct connecting the testis and epididymis. It is derived from the anterior portion of the wolffian duct (v. d. Broek, '07, '10 a, '10 b, '33).

Epididymis. The coils are the same size and appearance throughout. The epithelial lining of the epididymis consists, for the most part, of medium columnar cells with oval nuclei near the bases. Cilia and definite basal granules are present at the distal ends of the cells. Occasionally small pyramidal cells occur between the columnar cells.

Tests for spermatozoon motility were made in twenty-one cases. Fourteen of these showed spermatozoa with varying degrees of motility. In six cases numerous sperm were present in the epididymis, but were immotile. Three of these were animals which had been in captivity for a relatively long time, which may have affected the testis. No sperm were found in the epididymis of number 39, tested in December, in which no fully formed spermatozoa were found in the testis tubules.

Vas deferens. The epithelium consists of columnar cells with round or oval nuclei near the bases. These cells exhibit cilia with definite basal granules.

The wolffian duct in the pouch young of 13 days can be seen running parallel to the müllerian duct along the lateral edge of the mesonephros. The müllerian and wolffian ducts of each side join a short distance before opening into the urethra.

Urethra. The urethra is roughly circular in external outline, somewhat flattened on the dorsal side. The irregular lumen is eccentric in position, lying nearer the dorsal than the ventral side. Transitional epithelium, a continuation of that of the bladder, lines the whole length of the urethra. Beneath the epithelium is the tunica propria mucosae into which the glands extend. This layer blends imperceptibly with the submucosa. Peripheral to the glandular area and the small amount of connective tissue surrounding it are two layers of smooth muscle, an inner longitudinal and an outer circular layer. Surrounding this is a serosa.

The membranous part of the urethra is much narrower in diameter than the glandular portion (fig. 1). It consists of a mucosa, with transitional epithelium and tunica propria with a large amount of connective tissue, not separable from that of the submucosa. The muscle layers which are prominent in the glandular part here gradually become less regularly arranged and finally remain as bunches here and there of smooth muscle fibers. Toward the cavernous portion of the urethra may be found a few striated muscle fibers.

The glands in the enlarged portion of the urethra are all compound tubular with straight gland tubules (fig. 7). They occupy most of the area at the periphery of the urethra. Toward the lumen, the tubules join to form a large number of collecting ducts, which open separately into the urethra. The collecting ducts are lined with cuboidal or low columnar cells which stain lighter than the rest of the epithelium of the glands. The round or oval nuclei are placed in the center or at the bases of the cells. Farther from the lumen, the ducts branch and narrow and the cells change to the types characteristic of the gland tubules.

Three different types of prostate gland are found in the opossum, each with a distinct type of epithelium and each limited to a definite region of the urethra. The first type of gland is found in the orange region of the urethra (prostate I, fig. 1). The tubules are narrow and are separated by a fairly large amount of connective tissue (fig. 2). The cells

are low columnar or cuboidal, with round or oval, darkly staining nuclei near the bases. At the distal ends of the cells are long cilia, within which is a varying amount of secretion. Two types of granules are present in the cells in the region distal to the nucleus and in the secretion masses in the cilia.

The tubules of the second type of urethral gland occupy the pink area of the glandular urethra (prostate II in fig. 1). The tubules are separated from each other by an extremely small amount of connective tissue (fig. 3). The tubules have a larger diameter than the preceding type and the epithelial cells are higher and wider. At the distal part of the tubules, the cells are columnar with a more or less granular cytoplasm and with large masses of granular material protruding from them (fig. 3). Farther proximad, the cells become more regular in height and what masses protrude are of a homogeneous nature.

The tubules of the third type of gland are separated from each other, also, by a very small amount of connective tissue. The gland lumina are wide and the epithelial cells are cuboidal, with round nuclei near the bases. The cells are clear usually with only the cell boundaries and the nuclei stained (fig. 4). In the lumina are found globules of clear substance of the same appearance and staining reactions as the cytoplasm of the cells. In some individuals, a relatively undifferentiated condition was found in the gland cells.

Pouch young of various ages were studied in serial section in order to determine the origin and development of the prostate glands. Serial sections of pouch young of 13 days of age show small outgrowths from the epithelium of the urethral mucosa, beginning shortly posterior to the entrance of the wolffian duct into the urethra and extending on all sides of and for some distance along the urethra. No similar structures are found in the female. At 32 days (fig. 5) the buds are larger and some branching can be seen. With increasing age, the glands are found throughout a greater length of the urethra, being found at 46 days a short distance anterior

to the openings of the wolffian ducts. At 70 days, they are present even farther anterior. Branchings are apparent in specimens as young as 28 days and grow more complex with age. The tree-like character of these glands can be seen plainly at 70 days (fig. 6).

The name to be applied to glands of this region in marsupials has been a subject for discussion for some time. Cowper in 1704 stated that the urethra of the opossum is surrounded by "a Glandulous Body, Analogous to the Prostates in Men and other Creatures." Since that time, some authors have called these glands prostate and some urethral. Oudemans (1892) designated the glands as urethral glands because they were inside the smooth muscle layer of the urethra. However, although some authors accepted his terminology (Disselhorst, '04, '08; v. d. Broek, '07, '10 a, '33; Weber, '28), others continued to call the glands prostate (Sweet, '07; Osgood, '21; Hartman, '24; Engle, '26; Rotenberg, '28). A position midway between these two groups is taken by Fordham ('28) and Baecker ('30).

In deciding on the terminology to be used for these glands in marsupials, the anatomical relations, histology, and development should be considered. In the opossum, the ducts of these glands open into the urethra in the region immediately posterior to the bladder and around the openings of the vasa deferentia. They are thus similar in general position to the openings of the prostate glands in other mammals.

The position of the glands inside or outside the urethral muscles cannot be used in deciding upon the terminology because of the many transitional conditions which are found in mammals. Carnivores have a definite gland, part of which is surrounded by a separate capsule of muscle and part by the urethral muscle. In some Edentates, the prostate is outside, in others, inside the urethral muscle (Oudemans, 1892; Klinckowström, 1895; Wislocki, '28). In the sheep, the prostate is diffuse, with no separate capsule (Schmaltz, '11) and a similar condition is found in the hippopotamus (Hofman, '23). Baecker ('30) states that in the marsupial examined by

him, some of the glands break through the muscle layers and form flat glands outside the urethral muscle.

In early development these glands are similar to the prostate glands in other mammals (Pallin, '01; Eberth, '04; Stieve, '30; Price, '36). The difference arises later when the glands in most mammals penetrate the muscle layers of the urethra and form a separate organ, connected with the urethra by a duct or ducts, while the glands in the opossum remain within the walls of the urethra.

The glands resemble histologically the prostate glands of other mammals rather than the urethral glands. The former are said in most cases to be compound tubular glands with many branchings which agrees with the type found in marsupials. The urethral glands are small, scattered, compound acinous or tubular glands with few branchings (Rauther, '04; Hirsch, '26). In support of the idea that the glands in the marsupial cannot be urethral glands is the additional fact that urethral glands resembling those of other mammals have been found in the opossum (see Urethral glands, below).

It is, therefore, concluded that the glands of the cranial part of the urethra of the opossum should be called prostate. The name prostate gland will be used for these glands in the remainder of this paper and the three different types will be designated prostate I, II and III, proceeding from anterior to posterior.

Urethral glands. In the connective tissue of the membranous urethra throughout almost its entire length are found a few small glands, especially on the dorsal side. They are composed of groups of acini, with cuboidal or pyramidal cells resting on a basement membrane. The cytoplasm is granular with occasional vacuoles and small round nuclei may be seen in the basal half of the cells. Lumina of the acini are very narrow or may not be apparent at all. These glands are similar to the urethral glands described by Rauther ('04) in the guinea pig and by Hirsch ('26) in man.

Cowper's glands. The largest lobe of Cowper's gland (Cowper's 1, fig. 1) is surrounded by a layer of striated

muscle. Within this is a narrow layer of connective tissue, which extends between the gland tubules in thin strands. The smaller lobes (Cowper's 2 and 3, fig. 1) are enclosed in a common capsule of striated muscle, but have separate capsules of connective tissue. All three lobes are compound tubular with irregular lumina.

The histology of all three lobes varies with the degree of distension of the gland. In small glands, the epithelium is high columnar with oblong nuclei placed the long way of the cell, usually near the base; with greater distension, the cells may be so flattened as to appear as a narrow line of nuclei, separated from the nuclei of another tubule by a narrower line of connective tissue. Lobe 3 shows usually tall or medium columnar cells; lobe 2 has more flattened cells, while lobe 1 has very low cells with large lumina. No definite granules can be seen in the cells. The secretion fixed in the lumina is a homogeneous mass, staining evenly. The epithelium lining the large sinuses which are the ducts of Cowper's glands, is of a character similar to that of the glands themselves. This type continues until shortly before the sinuses join the urethra where there is a change to the transitional epithelium characteristic of the urethra.

In the pouch young, the anlagen of Cowper's glands are present in the 13-day-old specimen as single solid cords of cells on each side, extending dorsally and laterally from the urethra. With increasing age, the glands branch and the parts separate from each other. The tubules within each lobe branch to form a compound tubular gland. The lumina develop fairly early, extending at 70 days to a point somewhat beyond the first branchings of each lobe. The connection of all three parts remains in the adult as the common duct emptying into the urethra.

Homologs of Cowper's glands appear in the female at the same age as in the male. However, they do not branch as much and remain only as small structures. According to v. d. Broek ('10 b) they are non-functional rudiments in the adult.

EXPERIMENTAL TREATMENT

While the study of the gross and microscopical anatomy has shown that the male reproductive system of the opossum differs in many details from that of the placental mammals, the fact that it is essentially similar has been established. In order to determine whether the physiology was similar, an introductory study was made of the function of the testis. For this purpose, castrations were performed and gonadotropic hormones injected into semi-castrate animals.

Castration

Removal of the testes results in a variety of changes in mammals. In the rat, castration has been found to result in a decrease in weight of the prostate and seminal vesicles (Korenchevsky and Dennison, '34), and in cytological changes in these organs (Moore, Price and Gallagher, '30; Moore, Hughes and Gallagher, '30). Changes have been found to occur, also, in Cowper's glands in the rat (Heller, '30, '32). In other mammals, similar castration effects have been found, but the time required for them to become pronounced is much longer than in the rat (Leydolph, '29; Baker, '28; Heller, '32).

Castrations were performed in the opossum to determine if changes in the accessory glands occur comparable to those in other mammals. Ten animals were used for this experimental treatment. Castration was performed by removal of the entire scrotum with the enclosed testes and epididymides. The animals were sacrificed at varying lengths of time after operation and castration effects were studied on weight of the urethra and Cowper's glands and on the cytology of the prostate and Cowper's glands.

The procedure followed in removing organs for weighing was the same in all cases. The urethra was removed from the body cavity and the bladder cut off just anterior to the ureters. The posterior cut was made just anterior to the points where Cowper's glands join the urethra. The whole length of the urethra was then weighed. The weights of

Cowper's glands were obtained by separating Cowper's 1 from the adjoining tissue and cutting the duct where it joins the urethra. Cowper's 2 and 3 were removed in the same manner and weighed together.

The absolute weights of the organs of castrate and normal animals are given in table 1. To allow for the wide variation in body weight of the opossums, the weights of the organs per kilogram body weight were calculated. These figures are also given in table 1 and the conclusions in regard to weight changes are based upon these.

A striking decrease in relative weights of the accessory organs of the castrate animals is evident in comparing them with the normals. This weight change is found in all the organs weighed, but is more evident in the case of Cowper's 1, where the effect of castration is pronounced.

Reduction in diameter of the whole glandular urethra showed that all three types of prostate gland were affected by castration. However, each type showed a different degree of histological change.

Prostate I. The changes shown by the glands of this type varied with the length of castration and the individual. Twenty days after castration, the lumen of the urethra was enlarged and the glandular epithelium more or less disorganized. After 90 days of castration, changes were more apparent, although the degree of change was not the same in all specimens examined. Opossum 41 showed the lumen of the urethra to be decidedly wider than normal with a decrease in the width of the glandular layer. There was a disorganization of the tubules of the peripheral part of the urethra so that the epithelial character was lost (fig. 8), thus differing decidedly from the normal as represented by the 20-day semi-castrate (fig. 7). The cells of this region, where cell boundaries could be distinguished, were cuboidal, with the nuclei filling up most of the cell space. Granular inclusions were found in the cells and phagocytes were present. A few short cilia were seen attached to cells in the proximal parts of the tubules but no masses of secretion were found around them.

TABLE 1
Weights of normal and experimental animals

NUMBER	DATE	TREATMENT	BODY WEIGHT, KG.	ABSOLUTE WEIGHT, GM.			WEIGHT PER KILOGRAM BODY WEIGHT		
				Cowper's 2 & 3	Cowper's 1	Urethra	Cowper's 2 & 3	Cowper's 1	Urethra
Normal									
35	November	Normal	0.77	0.074	0.088		0.096	0.114	
36	November	Normal	2.15	0.112	0.078		0.052	0.036	
64	April	Normal	1.59	0.213	0.294	5.15	0.134	0.185	3.239
73	April	Normal	1.31	0.130	0.150	2.94	0.100	0.115	2.244
75	May	Normal	1.59	0.270	0.434	4.40	0.170	0.273	2.767
76	May	Normal	2.47	0.517	1.222	11.79	0.209	0.495	4.773
77	May	Normal	3.40	0.518	1.771	9.40	0.152	0.521	2.765
78	June	Normal	3.25	0.571	2.510	11.39	0.176	0.772	3.507
79	February	Normal	1.36	0.190	0.335	3.83	0.140	0.246	2.812
Castrate									
TIME CAST.									
84	April	7 days	2.92	0.390	1.900	6.76	0.133	0.650	2.312
82	March	10 days	2.18	0.290	1.750	5.19	0.133	0.801	2.376
37	December	20 days	2.72	0.123	0.111		0.045	0.041	
81	March	22 days	1.99	0.235	0.675	4.58	0.118	0.340	2.304
42	February	35 days	2.01	0.102	0.038	1.32	0.051	0.019	0.657
38	January	50 days	1.61	0.091	0.044		0.057	0.027	
39	February	65 days	1.19	0.050	0.024	0.28	0.042	0.020	0.239
41	April	90 days	2.69	0.098	0.178	1.92	0.037	0.066	0.714
49	May	90 days	1.65	0.131	0.146	1.53	0.080	0.088	0.927
83	June	90 days	2.24	0.135	0.105	1.53	0.060	0.047	0.682
Gonadotropic hormone injection									
DAILY INJ.									
45	February		1.05	0.212	0.415	5.44 ¹	0.202	0.395	5.181
46	February	25 RU prsp.	1.05	0.170	0.130	2.12 ¹	0.162	0.124	2.019
48	February	75 RU prsp.	1.08	0.324	1.278	8.59 ¹	0.300	1.183	7.954
47	February	50 RU Ant. S	1.08	0.504	1.428	7.27 ¹	0.467	1.322	6.731

¹ Weighed in alcohol and corrected. Corrected weight used in calculations.

Another 90-day castrate (opossum 49) showed a less disorganized condition, but one essentially similar to that of opossum 41. Most of the tubules were not patent. Those which were open showed cuboidal cells, in some of which granules were found near the distal ends. A few cells showed masses of secretion protruding from them. Phagocytes were present. Opossum 83, also castrate for 90 days, showed smaller tubules, with low cuboidal cells and no cilia.

Prostate II. Not so much change was apparent in the cells of these glands. After 20 days, the appearance was not different from that of a normal urethra of the same diameter. After castration for a longer period of time, an increase in the diameter of the lumen of the urethra was noticeable. The cells of the glands were cuboidal, with little or no secretion. At 90 days, the gland tubules were closed or were much narrower than usual, with an apparent increase in connective tissue. There were small masses attached to the free ends of some cells and secretion in some lumina.

Prostate III. Changes in the glands of this region were not apparent until after 50 days of castration. At this time the gland lumina were narrower and the gland cells granular instead of clear as they are normally. At 90 days, the tubules were narrower than normal, with an apparent increase in connective tissue. Some of the cells were granular throughout. A secretion appeared to be present in the lumina of some tubules.

Cowper's glands. The histological changes in Cowper's glands were not equally apparent in all lobes. No change was detectable in the smallest of the lobes, Cowper's 3, after 90 days of castration. Cowper's 2 showed no change until 65 days when the gland tubules were small with a large amount of connective tissue between them. The cells were low columnar with the nuclei occupying almost the entire cell. The same picture was found at 90 days. Cowper's 1 showed changes sooner than the other lobes. After 20 days of castration, the tubules were small and round, with a large amount

of connective tissue between them. At 65 days, the tubules were small and the nuclei almost completely filled the cells. There was some variation at 90 days, but in general there was a decrease in the diameter of the tubules with a relative increase in the amount of connective tissue. The cells were columnar. In opossum 49, some degenerating cells were seen in the lumina of some tubules, while secretion was present in others.

In general, castration changes were apparent in the lower weight of the urethra and of Cowper's glands as compared to the normal. Histological changes in the prostate glands were most noticeable in prostate gland I where a disorganization of gland tubules occurred so that the typical epithelial organization was lost after 90 days of castration. Phagocytes were present. Changes similar but not so extreme were found in prostates II and III.

Histological changes in Cowper's glands were most noticeable in Cowper's 1, the largest of the lobes. A decrease in the size of the gland tubules, with an apparent increase in connective tissue as a consequence, was seen. Changes were less apparent in Cowper's 2 and not detectable in Cowper's 3.

It follows therefore that, whereas castration changes appear in all parts of the prostate and Cowper's glands, the changes involve general cell involution which is somewhat slower than in such an animal as the rat. No especially distinctive cytoplasmic bodies exist which respond rapidly to testis removal.

Gonadotropic hormone administration

That the testis is not an autonomous organ but is under the control of some extra-testicular influence has been demonstrated in a number of mammals. The extent to which the opossum is similar was investigated by means of administration of gonad-stimulating substances to semi-castrate animals.

The four animals used for this injection series were matched as equally as possible in regard to body weight, and size and firmness of testes as palpated through the scrotum. Semi-castrations were performed by removing the testis and epi-

didymis of the left side through a scrotal incision. Injections were begun 36 hours after the operation and continued twice daily for 20 days. The animals were sacrificed on the day following the last injection.

Prospermin, a derivative of human male urine, was injected into two animals in doses of 25 rat units and 75 rat units daily. This was a preparation that showed marked testicular stimulation in the rat in doses of 5 rat units daily (unpublished observations of Carl R. Moore). An extract of human pregnancy urine, Antuitrin-S, was injected into the third animal, in amounts of 50 rat units daily, a fourth opossum served as a control and was not treated. Effects of injection were studied on weight of testes, urethra, and Cowper's glands, and on the histology of testes, prostate glands, and Cowper's glands.

The weight of the testes after treatment was compared with the weight of the testes removed before injections were begun. There was no significant weight change after injection as the increase in testicular weight in the injected animals was 18, 14 and 8%, while the control showed an increase of 14%. Histological examination showed the testes after injection to be no different from those removed before treatment.

In regard to the accessory glands, opossums 47 and 48, receiving respectively 50 rat units of Antuitrin-S and 75 rat units of prospermin daily, showed increase in relative weights of urethra and Cowper's glands (table 1). The other injected animal, which received 25 rat units of prospermin daily, showed weights of these organs within the range of normal variation as given in the table.

Histologically all four animals of this group showed the glands of the urethra packed closely together and widely distended, similar to the normal condition (figs. 2, 3, 4). There is no criterion for judging increased secretory activity beyond the maximal amount found in normal animals. Cowper's glands in opossum 46, which had received the smaller amount of prospermin showed no variation greater than the normal animals. Opossum 47, injected with Antuitrin-S, showed no

changes in Cowper's 2 and 3. Cowper's 1 in this animal was much distended, with cuboidal epithelial cells and the connective tissue between the tubules only a thin line. Opossum 48, which had received 75 rat units of prospermin daily, showed the same changes as above in Cowper's 2 and more distension, with consequently lower epithelium, in Cowper's 1 compared to the control.

Thus injections of these two gonadotropic substances in amounts representing huge daily dosages for the rat caused detectable changes in the accessory glands of the opossum.

DISCUSSION

The morphology of the male reproductive system of the opossum has been shown in this study to be essentially similar to that of placental mammals. Some differences occur, the most noteworthy being differences in the accessory reproductive glands. Ampullary glands and seminal vesicles are lacking. The prostate is greatly enlarged and is enclosed in the muscular capsule of the urethra. Cowper's glands are likewise enlarged and are separated into three paired lobes. However, even these peculiarities do not mean that the reproductive system of the opossum differs basically from that of placental mammals. The prostate gland, composed of three regions, each with a distinct type of epithelium, and Cowper's glands, despite the peculiarities exhibited by them, are yet like those of other mammals.

While the opossum breeds throughout most of the year (Hartman, '28), a period of anoestrus in November and December has been found in the female (Hartman, '23 a, '23 b, '28). A similar period might be supposed to occur in the male. However, Painter ('22) found mature sperm, and all stages leading up to their formation in the testes of four specimens of opossum examined in October, November and January. He found that the maturation stages were more abundant in the testes preserved in January than in those preserved in October and November. The presence of motile sperm in the epididymis has been shown in every month from November to June

in the present study. This, with the study of Painter ('22), would indicate that there is no anoestrous period in October, November and December.

The possibility of a lowered period of secretory activity of the testis with an effect on the activity of the secondary reproductive glands has not been ruled out. The fact that most of the larger and more distended glands were found in animals in the spring months seems to indicate a seasonal variation. However, one animal, sacrificed in December, possessed large glands. It is probable that the differences in size are due largely to age.

In the investigation of the physiology of the male reproductive tract of the opossum, the function of the testis in influencing the glands was investigated by means of castrations and by administration of gonadotropic hormones. As in other animals, castration effects were noted as decrease in weight of prostate and Cowper's glands (Leydolph, '29; Baker, '28; Korenchevsky and Dennison, '34; Heller, '32). The histological effects were evident as a degeneration of the prostate and Cowper's glands, being especially pronounced in prostate I and in Cowper's 1. Similar effects have been noted in the rat (Moore, Price, and Gallagher, '30; Heller, '32).

The function of the testis was studied by administration of gonadotropic hormones. In the rat it has been shown that normal animals injected with gonadotropic hormones show a stimulation of the testes as indicated by weight increase (Moore and Price, '32 a, '32 b; Morrell, '33; Moore, '36; Price, '36), this stimulation being especially pronounced in young animals. The increase in secretory activity of the testis as a result of injection is shown as an increase in size of accessory glands, also noted by the above authors. Wells ('35, '36) and Baker and Johnson ('36) have found in the sexually inactive ground squirrel an increase in testis size and activity and a stimulation of the accessories to the condition found in the reproductive season after injection of gonadotropic hormones.

The gonadotropic hormones, Antuitrin-S and prospermin, were injected into semi-castrate opossums in large doses. There was no significant change in weight of the testis after injection and no histological difference between the testis removed before and the one removed after treatment. This is in agreement with the results of Moore and Price ('32 b) and Moore ('36), who have shown that response of testes of animals treated with gonadotropic hormone decreases with age and that in the rat the response of mature animals is very slight.

A stimulation of the testis in the opossum to increased secretion of hormone was indicated by the increased weight of the urethra and Cowper's glands with Antuitrin-S and with the larger dose of prospermin administered. No change in histology of prostate or Cowper's glands was detectable.

SUMMARY AND CONCLUSIONS

1. A study of the male reproductive system of the opossum was conducted on forty-three specimens of *Didelphis virginiana* Kerr.

2. The urethra of the male opossum possesses large glands radially arranged around the lumen at the cranial portion of the urethra, just posterior to the neck of the bladder.

3. Because of their position, embryonic origin, and similarity of relationship to the glands of other mammals, these glands are designated as prostate glands.

4. Three definite types of prostate glands are present in the adult animal, each with a distinctive epithelium.

5. Small groups of acinous glands found in the membranous urethra have been designated urethral glands.

6. Cowper's glands are well developed in the opossum, being divided into three paired lobes.

7. Castration effects in the form of lowered weight and degeneration of gland tissue have been found in the prostate glands, being especially noted in prostate I.

8. Castration effects have been found, also, in the lobes of Cowper's gland, especially in the largest lobe. These changes

are evident as lowered weight and decrease in size of gland tubules.

9. Injections of Antuitrin-S and prospermin into adult male opossums effected no detectable change in weight or histology of the testes. With large doses, the urethra and Cowper's glands showed a greater weight than the normal. Histological changes were not detected in the accessory glands.

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PLATE 1

EXPLANATION OF FIGURES

- 2 Cells of prostate I from normal male. $\times 1117$. Bouin's, iron haematoxylin.
- 3 Cells of peripheral part of prostate II from normal male. $\times 1117$. Bouin's, iron haematoxylin.
- 4 Cells of prostate III from normal male. $\times 1117$. Bouin's iron haematoxylin.
- 5 Cross section of urethra of 32-day pouch young. $\times 51$. Bouin's, Ehrlich's acid haematoxylin.
- 6 Cross section of urethra of 70-day pouch young. $\times 51$. Bouin's, Ehrlich's acid haematoxylin.
- 7 Periphery of prostate I of 20-day semi-castrate. $\times 233$. Bouin's, Ehrlich's acid haematoxylin.
- 8 Periphery of prostate I of 90-day castrate. $\times 233$. Bouin's, Ehrlich's acid haematoxylin.

